

**WHAT IS CLAIMED:**

1. A method of increasing expression of native extracellular inducible microbial enzymes from *Trichoderma* or *Gliocladium* species, said method comprising:

5 culturing *Trichoderma* or *Gliocladium* species in a medium containing material sufficient to induce expression of native extracellular inducible microbial enzymes, low levels of carbohydrates, and/or low levels of reduced nitrogen compounds under conditions effective to increase expression of native fungal extracellular inducible microbial enzymes.

10 2. A method according to claim 1, wherein the medium further comprises a level of chitin containing material sufficient to induce expression of native extracellular inducible microbial enzymes.

15 3. A method according to claim 1, wherein said culturing is carried out with a low level of carbohydrates.

4. A method according to claim 1, wherein said culturing is carried out with a low level of reduced nitrogen compounds.

20 5. A method according to claim 1, wherein the medium further comprises a level of chitin containing material sufficient to induce expression of native extracellular inducible microbial enzymes and a low level of carbohydrates.

25 6. A method according to claim 1, wherein the medium further comprises a level of chitin containing material sufficient to induce expression of native extracellular inducible microbial enzymes and a low level of reduced nitrogen compounds.

30 7. A method according to claim 1, wherein said culturing is carried out with a low level of carbohydrates and a low level of reduced nitrogen compounds.

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8. A method according to claim 1, wherein the carbohydrates are present in the medium at a level of 5 to 50mM.

5 9. A method according to claim 1, wherein the reduced nitrogen compound is present in the medium at a level of 3 to 40mM.

10 10. A method according to claim 1, wherein the carbohydrates are selected from the group consisting of hexoses, pentoses, sugar alcohols, disaccharides, amino sugars, easily degraded  $\alpha$ -linked polymers, oligomers thereof, and mixtures thereof.

15 11. A method according to claim 1, wherein the carbohydrates are selected from the group consisting of glucose, galactose, mannose, fructose, xylose, ribose, mannitol, sorbitol, glucosamine, galactosamine, starch, sucrose, maltose, and mixtures thereof.

20 12. A method according to claim 1, wherein the reduced nitrogen compound is selected from the group consisting of ammonium salts, glutamine, urea, amino acids and mixtures thereof.

25 13. A method according to claim 1, wherein the extracellular inducible enzymes are selected from a group consisting of enzymes that degrade chitin,  $\beta$ -1,3 glucans, cellulose, hemicellulose, phytic acid, and proteins.

14. A method according to claim 13, wherein the extracellular inducible enzymes degrade chitin.

30 15. A method according to claim 13, wherein the extracellular inducible enzymes degrade  $\beta$ -1,3 glucans.

16. A method according to claim 13, wherein the extracellular inducible enzymes degrade cellulose.

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5                    18.     A method according to claim 13, wherein the extracellular  
inducible enzymes degrade phytic acid.

culturing fungi, which have been transformed with at least one transgene controlled by a promoter, in a medium containing an inducer of the promoter, high levels of carbohydrates, and/or high levels of reduced nitrogen compounds under conditions effective to repress expression of native proteins and to enhance expression of proteins encoded by the at least one transgene.

20                    21.        A method according to claim 19, wherein said culturing is  
carried out with a high level of carbohydrates.

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23. A method according to claim 19, wherein said culturing is carried out with an inducer of the promoter and a high level of carbohydrates.

24. A method according to claim 19, wherein said culturing is  
30 carried out with an inducer of the promoter and a high level of reduced nitrogen  
compounds.

25. A method according to claim 19, wherein said culturing is carried out with a high level of carbohydrates and a high level of reduced nitrogen compounds.

5 26. A method according to claim 19, wherein the carbohydrates are present in the medium at a level of 100mM to 500mM.

10 27. A method according to claim 19, wherein the reduced nitrogen compound is present in the medium at a level of 35mM to 500mM.

15 28. A method according to claim 19, wherein the carbohydrates are selected from the group consisting of hexoses, pentoses, sugar alcohols, disaccharides, amino sugars, easily degraded  $\alpha$ -linked polymers, oligomers thereof, and mixtures thereof.

20 29. A method according to claim 19, wherein the carbohydrates are selected from the group consisting of glucose, galactose, mannose, fructose, xylose, ribose, mannitol, sorbitol, glucosamine, galactosamine, starch, sucrose, maltose, and mixtures thereof.

25 30. A method according to claim 19, wherein the reduced nitrogen compound is selected from the group consisting of ammonium salts, glutamine, urea, amino acids, and mixtures thereof.

30 31. A composition comprising:  
a fungal source of an extracellular inducible microbial enzyme and  
a bacterial source of an extracellular inducible microbial enzyme.

32. A composition according to claim 31, wherein the fungal source is a filamentous fungus or a yeast.

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33. A composition according to claim 32, wherein the fungal source is *Trichoderma* spp., *Rhizopus* spp., *Aphanocladium* spp., *Coccidioides* spp., *Aspergillus* spp., *Hyprocrea* spp., *Candida* spp., or mixtures thereof.

34. A composition according to claim 31, wherein the bacterial source is *Aeromonas* spp., *Streptomyces* spp., *Serratia* spp., *Bacillus* spp., *Chromobacter* spp., *Vibrio* spp., *Pseudomonas* spp., *Pyrococcus* spp., *Aeromonas* spp., or mixtures thereof.

35. A method of releasing N-acetylglucosamine from a chitinous source comprising:  
treating a chitinous source with a composition comprising:  
a fungal source of a extracellular inducible microbial enzyme and  
a bacterial source of a extracellular inducible microbial enzyme, said treating being carried out under conditions effective to release N-acetylglucosamine from the chitinous source.

36. A method according to claim 35, wherein the fungal source is a filamentous fungus or a yeast.

37. A method according to claim 36, wherein the fungal source is *Trichoderma* spp., *Rhizopus* spp., *Aphanocladium* spp., *Coccidioides* spp., *Aspergillus* spp., *Hyprocrea* spp., *Candida* spp., or mixtures thereof.

38. A method according to claim 35, wherein the bacterial source is *Aeromonas* spp., *Streptomyces* spp., *Serratia* spp., *Bacillus* spp., *Chromobacter* spp., *Vibrio* spp., *Pseudomonas* spp., *Pyrococcus* spp., or mixtures thereof.

39. A method according to claim 35, wherein said treating is carried out under alkaline pH conditions effective to produce an oligomer of N-acetylglucosamine.

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40. A method of repeatedly degrading different chitinous substrates comprising:

- 5 (a) treating a first chitinous substrate with a composition comprising:  
a fungal source of a extracellular inducible microbial enzyme and/or  
a bacterial source of a extracellular inducible microbial enzyme, under conditions effective to produce chitin;
- 10 (b) terminating step (a);  
(c) treating a second chitinous substrate with the same composition used to carry out step (a) under conditions effective to produce chitin.

41. A method according to claim 40, wherein, after termination of step (c), repeating step (c) with the same compositions used to carry out step (c).

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42. A method according to claim 40, wherein the fungal source is a filamentous fungus or a yeast.

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43. A method according to claim 42, wherein the fungal source is *Trichoderma* spp., *Rhizopus* spp., *Aphanocladium* spp., *Coccidioides* spp., *Aspergillus* spp., *Hyprocrea* spp., *Candida* spp., or mixtures thereof.

25 44. A method according to claim 40, wherein the bacterial source is *Aeromonas* spp., *Streptomyces* spp., *Serratia* spp., *Bacillus* spp., *Chromobacter* spp., *Vibrio* spp., *Pseudomonas* spp., *Pyrococcus* spp., or mixtures thereof.

30 45. A method of increasing expression of native extracellular inducible microbial enzymes from *Trichoderma* or *Gliocladium* species, said method comprising:

culturing *Trichoderma* or *Gliocladium* species in a medium containing material sufficient to induce expression of native extracellular inducible microbial enzymes, a carbon source not repressive of expression of fungal extracellular inducible microbial enzymes, and/or low levels of reduced nitrogen compounds under conditions effective to increase expression of native fungal cell extracellular inducible microbial enzymes.

46. A method according to claim 45, wherein the medium further comprises a level of chitin containing material sufficient to induce expression of native cell extracellular inducible microbial enzymes.

47. A method according to claim 45, wherein said culturing is carried out with a carbon source not repressive to fungal extracellular inducible microbial enzymes.

48. A method according to claim 45, wherein said culturing is carried out with a low level of reduced nitrogen compounds.

49. A method according to claim 45, wherein the medium further comprises a level of chitin containing material sufficient to induce expression of native extracellular inducible microbial enzymes and a carbon source not repressive to fungal extracellular inducible microbial enzymes.

50. A method according to claim 45, wherein the medium further comprises a level of chitin containing material sufficient to induce expression of native extracellular inducible microbial enzymes and a low level of reduced nitrogen compound.

51. A method according to claim 45, wherein said culturing is carried out with a carbon source not repressive to expression of fungal extracellular inducible microbial enzymes and a low level of reduced nitrogen compound.

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53. A method according to claim 45, wherein the nitrogen  
5 compound is selected from the group consisting of ammonium salts, glutamine,  
urea, amino acids and mixtures thereof.

54. A method of enhancing purity of a heterologous recombinant protein expressed in a culture of fungi, said method comprising:

10 culturing the recombinant fungi in a medium containing nitrate as the medium's sole nitrogen source under conditions effective to induce expression of the heterologous protein while reducing expression of other proteins.